while up-regulating all other genes, suggesting a possible protective effect. Caspase-3, a key signaling molecule in apoptosis, was significantly up-regulated (12x FS) under Co alone. While previous studies showed increased apoptosis due to mechanical injury, we have now shown a direct link between chondrocyte apoptosis in normal cartilage due to co-culture with excised joint capsule.

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### MICRORNA ARE DIFFERENTIALLY EXPRESSED IN OSTEOARTHRITIC TISSUE

**S.W. Jones**, G.L. Watkins, S.M. Brockbank, M.R. Needham, P. Newham
*AstraZeneca, Macclesfield, United Kingdom*

**Purpose:** MicroRNA (miRNA) are short, non-coding RNA molecules, endogenously produced in mammalian cells and are believed to mediate protein translation. At present, around 300 human miRNA have been identified with each miRNA hypothetically targeting the translation of multiple genes. Previous studies have demonstrated both differential tissue biodistribution and disease-specific expression of miRNA suggesting that miRNA regulate important physiological and pathological processes. However, currently little research has been undertaken to identify miRNA associated with osteoarthritis (OA) or to determine the role of miRNA in the progression of this disease.
**Methods:** To investigate the role of miRNA in OA we profiled the expression of 157 human miRNA (Applied biosystems early access panel) in human cartilage and bone tissue from both normal (control) donors and OA donors. MiRNA were extracted from cartilage and bone tissue using Trizol and the miRNA expression quantified by 2-step Taqman PCR utilising RT stem loop primers (Applied Biosystems) for first strand synthesis, and normalised to 18S.
**Results:** In total, 28 miRNA were identified with differential expression between diseased and normal cartilage tissue. The most notable changes were miR-9, miR-25 and miR-98, which were upregulated by 8-, 8- and 23-fold respectively, and miR-107, miR-146 and miR-149, which were downregulated by 4-, 4-, and 27, fold respectively. Furthermore, in bone miR-27b, miR34c and miR-122a were upregulated by 5-, 21- and 9-fold respectively
**Conclusions:** Our results show that miRNA are differentially expressed in both OA cartilage and OA bone. Modulating the expression and/or activity of these differentially expressed miRNA may help to understand the role of these miRNA in OA and could lead to identifying novel targets for therapeutic intervention.

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### GLUCOSE UPTAKE AND GLUCOSE TRANSPORTER-1 EXPRESSION IN HUMAN CHONDROCYTES ARE DIFFERENTIALLY REGULATED BY HIGH AND LOW GLUCOSE CONCENTRATIONS

**S.C. Rosa**[1], F. Judas[2], M.C. Lopes[1], A.F. Mendes[1]
*[1]Faculty of Pharmacy and Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [2]Orthopedics Department and Bone Bank, University Hospital of Coimbra and Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

**Purpose:** Chondrocytes utilize glucose both for energy production and plastic functions, including the synthesis of matrix components. Increasing evidence suggests that alterations in ambient glucose concentrations outside of normoglycemic ranges, as occurs in poorly controlled diabetes mellitus, can significantly impair chondrocyte anabolic functions. This is in agreement with recent studies showing a correlation between metabolic dysfunction, nutrient imbalance, diabetes mellitus and OA. Glucose uptake and delivery to metabolic and synthetic pools is, thus, crucial for chondrocyte functions. This work aimed at elucidating whether alterations in the extracellular glucose concentration affect its transport into chondrocytes, namely by elucidating the effects of those alterations on the expression of the glucose transporter-1 (GLUT-1) and on the overall glucose uptake capacity.
**Methods:** Human chondrocytes were isolated from the cartilage of the femoral condyles of cadaveric tissue donors without macroscopic signs of osteoarthritis. Subconfluent chondrocyte cultures were set up and serum starved for 16 hours after an initial 24h recovery period in medium supplemented with 5% FBS. Then, the cells were incubated in media containing different glucose concentrations (0, 5, 10 and 30 mM D-glucose) in the presence or absence of 4$\mu$M diphenyliodonium chloride (a NADPH oxidase inhibitor), for 48 hours. In another set of experiments and to further characterize the role of reactive oxygen species (ROS) on GLUT-1 expression, the chondrocyte cultures in regular medium (10 mM glucose) were treated with $H_2O_2$, 100$\mu$M or IL-1$\beta$, 30ng/ml. Total GLUT-1 content in whole cell lysates was evaluated by Western blot. Glucose uptake was measured as the amount of non-metabolizable 2-Deoxy-D-2-[2,6-$^3$H]glucose transported into chondrocytes for 30 minutes at 37°C.
**Results:** Glucose deprivation for 48 hours significantly increased total GLUT-1 protein levels (129,5$\pm$3,4%) and 2-Deoxy-D-Glucose uptake (114,2$\pm$5%) relatively to cells cultured with either 5 or 10 mM glucose. In contrast, GLUT-1 content (79,1$\pm$9,1%) and 2-Deoxy-D-Glucose uptake (72,3$\pm$3%) decreased in cells cultured for 48 hours under high glucose concentrations (30 mM). Furthermore, for each glucose concentration tested, addition of diphenyliodonium chloride further increased GLUT-1 protein levels. Further emphasizing the role of reactive oxygen species, treatment of chondrocyte cultures under normal glucose concentration with $H_2O_2$ reduced GLUT-1 content to 75,8$\pm$2,1%, an effect similar to that observed with 30mM glucose. On the opposite, IL-1$\beta$ increased GLUT-1 protein levels to 164$\pm$4,1% of those found in cells cultured under normal glucose conditions (10 mM).
**Conclusions:** Chondrocytes are capable of regulating GLUT-1 expression and the rate of glucose transport in response to alterations in the extracellular glucose concentration. Both GLUT-1 protein levels and glucose uptake are upregulated in response to glucose deprivation, what can maximize the ability of the cell to capture as much glucose as possible. The opposite occurs as the available glucose augments, which may constitute a defense mechanism against deleterious effects of increased intracellular glucose levels such as production of glucose-derived ROS. This is in agreement with the effects observed with $H_2O_2$ and with the antioxidant diphenyliodonium chloride, indicating that ROS mediate high glucose-induced downregulation of GLUT-1 levels and glucose uptake.

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### INCREASE IN THE GENE EXPRESSION OF MMP 13 AND BMP 2 IN HUMAN ARTHROTIC CARTILAGE CELLS AFTER THE TREATMENT WITH 5 AZA DEOXY CYTIDINE

**G. Gruber Jr.**[1], F.C. Fuerst[2], B. Yazdani-Biuki[2], M. Stradner[2], H. Clar[1], D. Setznagel[2], H. Angerer[2], W. Graninger[2], R. Windhager[1]
*[1]Medical University, Orthopaedic Surgery, Graz, Austria; [2]Medical University, Rheumatology, Graz, Austria*

**Purpose:** In Osteoarthritis the matrix metalloproteinases (MMPs) are important for collagen degradation and are suspects in the development of this disease. Especially MMP- 13 is present in cartilage and thought to be one of the major collagen proteinases.BMP-2 (bone morphogenic protein 2) is an impor-